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<u>L6</u>	Aequorea victoria	8782	<u>L6</u>
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L3: Entry 1 of 5

File: USPT

Aug 24, 2004

DOCUMENT-IDENTIFIER: US 6780975 B2

TITLE: Long wavelength engineered fluorescent proteins

Detailed Description Text (5):

In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, amino acid substitution is:

Detailed Description Text (26):

In another aspect this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at M42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222, or V224, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein.

Detailed Description Text (87):

Aequorea-related fluorescent proteins include, for example and without limitation, wild-type (native) Aequorea victoria GFP (D. C. Prasher et al., "Primary structure of the Aequorea victoria green fluorescent protein," Gene, (1992) 111:229-33), whose nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) are presented in FIG. 3; allelic variants of this sequence, e.g., Q80R, which has the glutamine residue at position 80 substituted with arginine (M. Chalfie et al., Science, (1994) 263:802-805); those engineered Aequorea-related fluorescent proteins described herein, e.g., in Table A or Table F, variants that include one or more folding mutations and fragments of these proteins that are fluorescent, such as Aequorea green fluorescent protein from which the two amino-terminal amino acids have been removed. Several of these contain different aromatic amino acids within the central chromophore and fluoresce at a distinctly shorter wavelength than wild type species. For example, engineered proteins P4 and P4-3 contain (in addition to other mutations) the substitution Y66H, whereas W2 and W7 contain (in addition to other mutations) Y66W. Other mutations both close to the chromophore region of the protein and remote from it in primary sequence may affect the spectral properties of GFP and are listed in the first part of the table below.

Detailed Description Text (88):

Additional mutations in Aequorea-related fluorescent proteins, referred to as "folding mutations," improve the ability of fluorescent proteins to fold at higher temperatures, and to be more fluorescent when expressed in mammalian cells, but have little or no effect on the peak wavelengths of excitation and emission. It should be noted that these may be combined with mutations that influence the spectral properties of GFP to produce proteins with altered spectral and folding properties. Folding mutations include: F64L, V68L, S72A, and also T44A, F99S,

h e b b g e e e f c e f

e ge

Y145F, N146I, M153T or A, V163A, I167T, S175G, S205T and N212K.

Detailed Description Text (98):

In another embodiment, an amino acid that is close to a second amino acid within about 0.5 nm of the chromophore can, upon substitution, alter the electronic properties of the second amino acid, in turn altering the electronic environment of the chromophore. Table D presents two such amino acids. The amino acids, L220 and V224 are close to E222 and oriented in the same direction in the .beta.-pleated sheet.

Detailed Description Text (100):

One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein. Preferably, the substitution at E222 is selected from the group of N and Q. The E222 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at F64.

Detailed Description Text (164):

The mutations F64L, V68L and S72A improve the folding of GFP at 37.quadrature. (B. P. Cormack et al. Gene 173:33 (1996)) but do not significantly shift emission spectra.

Detailed Description Paragraph Table (3):

TABLE B Original position and presumed role Change to Codon L42 Aliphatic residue near C.dbd.N of chromophore CFHLQRWYZ 5'YDS 3' 3'RHS 5' V61 Aliphatic residue near central --CH.dbd. of chromophore FYHCLR YDC RHg T62 Almost directly above center of chromophore bridge AVFS KYC MRg DEHKNQ VAS BTS FYHCLR YDC RHg V68 Aliphatic residue near carbonyl and G67 FYHL YWC RWg N121 Near C--N site of ring closure between T65 and G67 CFHLQRWYZ YDS RHS Y145 Packs near tyrosine ring of chromophore WCFL TKS AMS DEHNKQ VAS BTS H148 H-bonds to phenolate oxygen FYNI WWC WWg KQR MRg KYC V150 Aliphatic residue near tyrosine ring of chromophore FYHL YWC RWg F165 Packs near tyrosine ring CHQRWYZ YRS RYS I167 Aliphatic residue near phenolate; I167T has effects FYHL YWC RWg T203 H-bonds to phenolic oxygen of chromophore FHLQRWYZ YDS RHS E222 Protonation regulates ionization of chromophore HKNQ MAS KTS

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